Serial No.: 10/048,186 Filed: June 19, 2002

Page : 2 of 12

# Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

# **Listing of Claims:**

1. (currently amended) A bacterial host cell that produces a heterologous metabolite, the host cell comprising a nucleic acid sequence comprising a promoter and nucleic acid sequence encoding a biosynthetic enzyme for production of an isoprenoid, a polyketide, or a polyhydroxyalkanoate the heterologous metabolite; the nucleic acid sequence being operably linked to the promoter which is controlled bound by ntrC a response regulator protein; the host cell being genetically modified by deletion or inactivating mutation in glnL of a gene encoding a histidine protein kinase having specificity for the response regulator protein such that the promoter is regulated by acetyl phosphate in the absence of nitrogen starvation, wherein the bacterial host cell is an E. coli cell.

#### 2.-4. (cancelled)

- 5. (currently amended) The host cell of claim [[4]]  $\underline{1}$  wherein the promoter is glnAp2.
  - 6.-9. (cancelled)

Serial No.: 10/048,186 Filed: June 19, 2002

Page : 3 of 12

10. (currently amended) The host cell of claim 9 wherein the metabolite is An E. coli host cell comprising a first expression cassette comprising a promoter and a nucleic acid sequence encoding a first enzyme that catalyzes biosynthesis of an isoprenoid; the nucleic acid sequence being operably linked to the glnAp2 promoter which is regulated by acetyl phosphate in the absence of nitrogen starvation; and a nucleic acid sequence encoding a second enzyme that catalyzes biosynthesis of the isoprenoid.

- 11. (withdrawn) The host cell of claim 10 wherein the isoprenoid is a carotenoid.
- 12. (original) The host cell of claim 10 wherein the isoprenoid is lycopene, β-carotene, astaxanthin, or one of their precursors.
- 13. (original) The host cell of claim 10 wherein the first enzyme is isopentenyl diphosphate isomerase, geranylgeranyl diphosphate synthase, or 1-deoxyxylulose 5-phosphate synthase.

### 14.-16. (cancelled)

17. (currently amended) The host cell of claim [[9]] 10 wherein the cell is lacking a functional glnL histidine protein kinase gene.

# 18.-20. (cancelled)

21. (currently amended) The host cell of claim 10 wherein the host cell further contains a second expression cassette comprising a nucleic acid sequence encoding a phosphoenolpyruvate synthase operably linked to a promoter which is regulated by acetyl phosphate concentration.

Serial No. : 10/048,186
Filed : June 19, 2002

Page : 4 of 12

22. (withdrawn) A method of producing a heterologous isoprenoid in a host cell, the method comprising:

providing the host cell of claim [[9]] 10, wherein the first enzyme is a biosynthetic enzyme that catalyzes synthesis of the heterologous isoprenoid;

overexpressing a heterologous phosphoenolpyruvate synthase; and expressing biosynthetic enzymes required for synthesis of the heterologous isoprenoid.

23. (withdrawn) A method of producing a lycopene in a bacterial host cell, the method comprising:

providing the host cell of claim 10; and

expressing a heterologous 1-deoxy-D-xylulose 5-phosphate synthase, a heterologous geranylgeranyl diphosphate synthase, a heterologous phytoene synthase, and a heterologous phytoene desaturase, at least one of which is the first enzyme expressed from the first expression cassette expressed from a coding nucleic acid whose transcription is controlled by ntrC and acetyl phosphate concentration.

24. (currently amended) A kit comprising (i) a nucleic acid sequence containing a promoter controlled by a response regulator protein bound by ntrC such that the promoter is regulated by acetyl phosphate in a defined bacterial host cell, and a coding sequence that encodes an enzyme for isoprenoid biosynthesis; and (ii) the defined host cell which is an *E. coli* host cell genetically modified by deletion or inactivating mutation of a histidine protein kinase glnL gene.

### 25.-27. (cancelled)

28. (withdrawn) The nucleic acid sequence of claim [[27]] 33 wherein the isoprenoid is a carotenoid.

Serial No.: 10/048,186 Filed: June 19, 2002

Page : 5 of 12

29. (currently amended) The nucleic acid sequence of claim [[28]]  $\underline{33}$  wherein the isoprenoid is lycopene,  $\beta$ -carotene, astaxanthin, or one of their precursors.

30.-32. (cancelled)

- 33. (currently amended) The nucleic acid sequence of claim 31 A nucleic acid sequence comprising a promoter and a sequence encoding a biosynthetic enzyme required for the production of an isoprenoid, wherein the promoter is bound by ntrC and functional in an *E. coli* cell.
  - 34. (original) The nucleic acid sequence of claim 33 wherein the promoter is glnAp2.
- 35. (currently amended) The nucleic acid sequence of claim [[27]] 33 wherein the biosynthetic enzyme is isopentenyl diphosphate isomerase, geranylgeranyl diphosphate synthase, or 1-deoxyxylulose 5-phosphate synthase, or phosphoenolpyruvate synthase.
  - 36. (cancelled)
- 37. (withdrawn) The host cell of claim 1 wherein the heterologous metabolite is a polyketide.
- 38. (withdrawn) The host cell of claim 1 wherein the heterologous metabolite is a polyhdroxyalkanoate polyhydroxyalkanoate.
  - 39. (cancelled)

Serial No. : 10/048,186 Filed : June 19, 2002

Page : 6 of 12

40. (previously presented) A E. coli bacterial host cell comprising:

- (i) a genetic alteration inactivating the glnL gene; and
- (ii) a nucleic acid sequence comprising a coding sequence encoding a biosynthetic enzyme that catalyzes production of an isoprenoid, polyketide, or polyhdroxyalkanoate, and an operably linked promoter that is regulated bound by ntrC and acetyl phosphate.
- 41. (previously presented) The host cell of claim 40 wherein the biosynthetic enzyme is isopentenyl diphosphate isomerase, geranylgeranyl diphosphate synthase, 1-deoxyxylulose 5-phosphate synthase, or phosphoenolpyruvate synthase.
  - 42. (cancelled)
  - 43. (cancelled)
  - 44. (cancelled)
- 45. (previously presented) The kit of claim 42 wherein the promoter is the glnAp2 promoter.
- 46. (withdrawn) A method of producing a heterologous metabolite in a bacterial host cell, the method comprising:

providing the host cell of claim 1; and culturing the host cell under conditions such that acetyl phosphate triggers the promoter.

47. (withdrawn) The method of claim 46 in which the culturing comprises nitrogen rich conditions.

Serial No.: 10/048,186
Filed: June 19, 2002

Page : 7 of 12

48. (withdrawn) The method of claim 46 in which the culturing comprises growth to late logarithmic growth or stationary phase.

- 49. (new) The method of claim 46 in which the culturing comprises growth to stationary phase.
- 50. (new) The method of claim 48 in which the metabolite is lycopene, the promoter is glnAp2, and at least 5 mg L<sup>-1</sup> of lycopene are produced.
  - 51. (new) The nucleic acid sequence of claim 33 wherein the isoprenoid is lycopene.
  - 52. (new) The host cell of claim 10 wherein the isoprenoid is lycopene.